

CLAIMS

1 An isolated nucleic acid which comprises a nucleotide
5 sequence which encodes a sugar-signalling transcription factor
which is capable of activating a promoter of a gene encoding an
enzyme involved in the synthesis or deposition of starch .

2 A nucleic acid as claimed in claim 1 wherein the
10 transcription factor is a WRKY protein which is capable of
activating the promoter within a plant in response to sugar levels
in the plant

3 A nucleic acid as claimed in claim 2 wherein the promoter
15 comprises at least one SURE element and/or W box element to which
the transcription factor binds

4 A nucleic acid as claimed in claim 3 wherein the promoter is
selected from the list consisting of: *isol*, *sbe1*, *sbeIIb*, *ssI*,
20 *agpaseS*.

5 A nucleic acid as claimed in any one of the preceding claims
claim 1 wherein the nucleotide sequence is a *susiba2* nucleotide
sequence which:

25 (i) encodes the *SUSIBA2* polypeptide given in Figure 1, or
(ii) encodes a variant *SUSIBA2* polypeptide which is a variant of
the *SUSIBA2* amino acid sequence given in Figure 1 and which shares
at least about 50%, 60%, 70%, 80% or 90% identity therewith,

30 6 A nucleic acid as claimed in claim 5 wherein the nucleotide
sequence:

(i) consists of the barley *susiba2* coding sequence given in Figure
1 or one which is degeneratively equivalent thereto,
(ii) comprises a wheat or rice *susiba2* coding sequence given in the
35 Sequence Annex, or one which is degeneratively equivalent to

either.

7 A nucleic acid as claimed in claim 5 wherein the susiba2
nucleotide sequence encodes a derivative of a susiba2 coding
5 sequence of claim 6 by way of addition, insertion, deletion or
substitution of one or codons.

8 A nucleic acid as claimed in claim 5 wherein the susiba2
nucleotide sequence consists of an allelic or other homologous or
10 orthologous variant of the barley susiba2 coding sequence given in
Figure 1.

9 An isolated nucleic acid which comprises a nucleotide
sequence which is the complement of the transcription factor-
15 encoding nucleotide sequence of any one of claims 1 to 8.

10 An isolated nucleic acid for use as a probe or primer, said
nucleic acid having a distinctive sequence of at least about 16-24
nucleotides in length, which sequence is present in Fig 1 or a
20 sequence which is degeneratively equivalent thereto, or the
complement of either.

11 An isolated nucleic acid as claimed in claim 10 wherein the
distinctive sequence encodes all or part of the SUSIBA2-specific
25 sequence:

ppmknavvhqinsnmpssigggmmracearnytnqysqaa

12 A process for producing a nucleic acid as claimed in claim 7
30 comprising the step of modifying a nucleic acid as claimed in claim
6.

13 A method for identifying or cloning a nucleic acid as claimed
in claim 6 or claim 8, which method employs a nucleic acid probe or
35 primer as claimed in claim 10 or claim 11.

14 A method as claimed in claim 13, which method comprises the steps of:

- (a) providing a preparation of nucleic acid from a plant cell;
- 5 (b) providing a nucleic acid molecule which is a nucleic acid probe or primer as claimed in claim 10 or claim 11,
- (c) contacting nucleic acid in said preparation with said nucleic acid molecule under conditions for hybridisation, and,
- (d) identifying nucleic acid in said preparation which hybridises
- 10 with said nucleic acid molecule.

15 A method as claimed in claim 13, which method comprises the steps of:

- (a) providing a preparation of nucleic acid from a plant cell;
- 15 (b) providing a pair of nucleic acid molecule primers suitable for PCR, at least one of said primers being a nucleic acid primer as claimed in claim 10 or claim 11,
- (c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,
- 20 (d) performing PCR and determining the presence or absence of an amplified PCR product.

16 A recombinant vector which comprises the nucleic acid of any one of claims 1 to 8.

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17 A vector as claimed in claim 16 wherein the nucleic acid is operably linked to a promoter for transcription in a host cell, wherein the promoter is optionally an inducible promoter.

30 18 A vector as claimed in claim 16 or claim 17 which is a plant vector.

19 A method which comprises the step of introducing the vector of any one of claims 16 to 18 into a host cell, and optionally

35 causing or allowing recombination between the vector and the host

cell genome such as to transform the host cell.

20 A host cell containing or transformed with a heterologous vector of any one of claims 16 to 18.

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21 A method for producing a transgenic plant, which method comprises the steps of:

(a) performing a method as claimed in claim 20 wherein the host cell is a plant cell,

10 (b) regenerating a plant from the transformed plant cell.

22 A transgenic plant which is obtainable by the method of claim 17, or which is a clone, or selfed or hybrid progeny or other descendant of said transgenic plant, which in each case includes a
15 heterologous nucleic acid of any one of claims 1 to 8.

23 A transgenic plant as claimed in claim 22 which is a seed crop plant.

20 24 A part of propagule from a plant as claimed in claim 22 or claim 23, which in either case includes a heterologous nucleic acid of any one of claims 1 to 8.

25 An isolated polypeptide sugar-signalling transcription factor
25 which is encoded by the nucleotide sequence of any one of claims 1 to 8.

26 A polypeptide as claimed in claim 25 which is the SUSIBA2 polypeptide shown in Fig 1.

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27 A polypeptide which comprises the antigen-binding site of an antibody having specific binding affinity for the polypeptide of claim 26.

35 28 A method for activating the promoter of a gene encoding an

enzyme involved in the synthesis or deposition of starch in a plant,

wherein the promoter is activated by a sugar-signalling transcription factor,

5 which method comprises the step of causing or allowing expression of a heterologous nucleic acid as claimed in any one of claims 1 to 8 within the cells of the plant, thereby expressing the transcription factor therein.

10 29 A method as claimed in claim 28 which is preceded by the earlier step of introduction of the heterologous nucleic acid into a cell of the plant or an ancestor thereof.

30 A method for modulating the activity of a promoter of a gene
15 encoding an enzyme involved in the synthesis or deposition of starch in a plant,

wherein the promoter is activated by a sugar-signalling transcription factor,

which method comprises any of the following steps of:

- 20 (i) introducing all or part of a nucleic acid as claimed in claim 9 in the plant such as to reduce transcription factor expression by an antisense ODN mechanism;
- (ii) causing or allowing transcription from part of a nucleic acid as claimed in any one of claims 1 to 8 such as to reduce
- 25 transcription factor expression by co-suppression;
- (iii) use of nucleic acid encoding a ribozyme specific for a nucleic acid as claimed in any one of claims 1 to 8,
- (iv) use of double-stranded RNA which comprises an RNA sequence encoding part of the polypeptide of claim 25, which is optionally a
- 30 siRNA duplex consisting of between 20 and 25 bps.

31 A method of producing modified starch anabolism activity in plant comprising use of a method of any one of claims 28 to 30, and optionally recovering starch from the plant.

32 A method of binding, activating, or identifying a promoter which includes at least one SURE element and/or W box element, which method employs the step of contacting said promoter with a polypeptide of claim 25.

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33 A method of investigating or confirming whether a *cis* promoter element is present in a plant transcription factor consensus sequence in a target gene promoter, the method comprising:

- 10 (i) observing the expression of a reporter gene operably linked to the promoter in a plant cell in which the transcription factor is present,
- (ii) introducing into the plant cell a double stranded oligodeoxynucleotide (ODN) decoy corresponding to the promoter
- 15 element into the cell,
- (iii) observing the expression of the reporter gene in the presence of the ODN decoy,

wherein a reduction in expression from (i) to (iii) confirms that the plant transcription factor binds the promoter element.

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34 A method as claimed in claim 33 wherein the promoter element is a SURE element.

35 A method as claimed in claim 33 or claim 34 wherein the

25 promoter is the *isol* promoter.

36 A method as claimed in any one of claims 33 to 35 wherein the transcription factor is SUSIBA2.

30 37 A method as claimed in any one of claims 33 to 36 wherein the method is performed by transient expression assays of GFP reporter gene fluorescence in transformed barley endosperm cells.